



**Mast Group Ltd.**  
Mast House, Derby Road,  
Bootle, Merseyside, L20 1EA  
United Kingdom  
Tel: + 44 (0) 151 472 1444  
Fax: + 44 (0) 151 944 1332  
email: sales@mast-group.com  
Web: www.mast-group.com



**Mast Diagnostica GmbH**  
Feldstrasse 20  
DE-23858 Reinfeld  
Germany  
Tel: + 49 (0) 4533 2007 0  
Fax: + 49 (0) 4533 2007 68  
email: mast@mast-diagnostica.de  
Web: www.mast-group.com

**Mast Diagnostic**  
12 rue Jean-Jacques Mention  
CS91106, 80011 Amiens, CEDEX 1  
France  
Tél: + 33 (0) 3 22 80 80 67  
Fax: + 33 (0) 3 22 80 99 22  
email: info@mast-diagnostic.fr  
Web: www.mast-group.com



**Mast  
Group**

## MAST® ASSURE ANTISERUM VIBRIO CHOLERAЕ

### Intended Use

Liquid stable antisera for serotyping of *Vibrio cholerae* O1 and O139.

FOR IN VITRO DIAGNOSTIC USE ONLY

**Contents:** See pack label.

### Principle of the test

When the antiserum is mixed with a strain of *V. cholerae* which has antigens homologous with those in the antisera, the antigen and antibody will produce macroscopic agglutination. Absence of homologous antigen and antibody will produce no agglutination. This reaction with a combination of polyvalent and monovalent antisera is used to determine the serotype of the organism strain.

### Formulation

MAST® ASSURE ANTISERUM are prepared from rabbits hyperimmunised with standard strains of killed organisms possessing known serotypes or group specific antigens and contain 0.085% sodium azide as preservative.

### Stability and storage

Store unopened at 2 to 8°C until the expiry date shown on the pack label. Once opened, MAST® ASSURE ANTISERUM should be stored at 2 to 8°C and may be used until the expiry date given on the label. **Do not freeze reagents.**

### Warnings and precautions

For *in vitro* diagnostic use only. Observe approved biohazard precautions and aseptic techniques. To be used only by adequately trained and qualified laboratory personnel. Sterilise all biohazard waste before disposal. Sodium azide preservative may be toxic if ingested and may react with lead and copper plumbing to form highly explosive salts. Always dispose of by flushing to drain with plenty of water. Refer to Product Safety Data sheet.

### Materials required but not provided

Standard microbiological supplies and equipment such as loops, micropipettes, applicator sticks, clean glass microscope slides or glass test tubes swabs, MAST® culture media, incinerators and incubators, etc., as well as reagents and additives such as sterile 0.85% saline solution.

### Procedure

#### Slide agglutination of live organisms

1. Dispense two 5 to 10µl volumes of sterile 0.85% saline solution (saline) onto a carefully cleaned microscope slide. The slide may be partitioned using a chinagraph pencil. With a platinum wire or disposable inoculation loop take one 1 to 2mm colony of live organisms from a fresh culture on Nutrient Agar and emulsify into each drop of saline to produce a distinct and uniform turbidity.
2. Place a drop (30 to 40µl) of antiserum onto one of the emulsified isolates and on to the other a drop (30 to 40µl) of saline as a control.  
**Note:** Do not allow the organism to contaminate the antiserum dropper bottle.
3. Mix the reagents by tilting the slide back and forth for 60 seconds while viewing it under indirect light against a dark background.
4. Distinct clumping or agglutination within this period, without clumping in the saline control (auto-agglutination), should be regarded as a positive result.

### Interpretation of results

- Isolates producing a distinct positive reaction with the polyvalent antisera are assumed to be *V. cholerae* O1.
- The *V. cholerae* polyvalent antiserum contains agglutinins for Factors A, B and C. *V. cholerae* serovar Ogawa antiserum contains agglutinins for Factor B. *V. cholerae* serovar Inaba antiserum contains agglutinins for Factor C.

- Further testing of the isolate should be conducted as described in steps 1 to 3, with monovalent antisera. Specimens that show agglutination only with Inaba-type serum should be reported as *V. cholerae* O1 serovar Inaba and specimens that show agglutination only with Ogawa-type serum should be reported as *V. cholerae* O1 serovar Ogawa. Specimens that show agglutination with both types of serum should be reported as *V. cholerae* O1 serovar Hikojima (which contains antigens for both Factors B and C) and gives a strong reaction with both Ogawa and Inaba antisera. The Ogawa-type strain may produce a trace of Factor C and give a slight reaction with Inaba antiserum.
- The serovar Inaba antiserum may show a delayed reaction with the polyvalent antiserum compared to the serovar Ogawa antiserum.
- Serotyping with live cells may not be possible with some strains of *V. cholerae* O1. Negative results with polyvalent antiserum, or where a polyvalent antiserum shows a positive and the monovalent a negative result, should be retested by heating an antigen suspension as directed below. Where heated antigen of a strain gives a negative result with polyvalent antiserum it is to be identified as a non-O1 *V. cholerae* strain.  
Suspend 3 to 5 times the size of a match head of organism in 3ml of physiological saline and heat to 121°C for 15 minutes or 100°C for 1 hour. Centrifuge the heated solution at 900g for 20 minutes, discard the supernatant, suspend the pellet in 0.5ml physiological saline and use as the heated cell suspension.
- *V. cholerae* O140 (referred to as serogroup Hakata) possesses Factor C and D and is regarded as an Inaba-type, based on the serotyping test.
- Some strains of *V. fluvialis* are reported to possess Factor C. Marine *Vibrio* bioserogroup 1875 is reported to possess either Factor B or C. These strains are distinguishable from *V. cholerae* in biochemical tests.
- Specimens that show agglutination only with O139 Bengal serum should be reported as *V. cholerae* O139 Bengal.

**Note:-** it should be remembered that El Tor *Vibrios* cannot be distinguished from *V. cholerae* O1 by serological means.

### Limitations of use

Only cultures of organisms identified as *V. cholerae* by morphological and biochemical features should be serotyped with this product. Polyvalent antisera are intended for use in rapid slide agglutination tests only. Monovalent antisera are intended for use in rapid slide agglutination tests for further identification. Positive results may be confirmed by tube agglutination tests.

### Quality control

Quality control should be performed with at least one organism to demonstrate a positive reaction and at least one organism to demonstrate a negative reaction. Do not use the product if the reactions with the control organisms are incorrect. Check for signs of deterioration. Do not use reagents if they are contaminated or cloudy.

### Performance

#### 1. Sensitivity

When 1 drop of antiserum is allowed to react on a slide with a known serotype of a reference strain, granular macroscopic agglutination is observed.

#### 2. Specificity

In tests performed as described, the respective antisera only react with reference strains corresponding to the antigens specified, while strains with non-corresponding antigens show no macroscopic agglutination.

### References

Bibliography available on request.